Original Research Article

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Existence of Yersinia Species in Marketable Milk and Some Dairy Products

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Abstract

Yersinia species are members of the *Enterobacteriaceae*. These bacteria have Gram-negative or Gram-variable, rod-shaped cells. *Yersinia* regards as one of the most important food-borne agents along with ability to grow at room and refrigerator temperatures. There are several intestinal and extraintestinal clinical symptoms caused by these organisms, appearing through mild gastroenteritis, mesenteric lymphadenitis, and pseudo appendicitis. Yersiniosis is a zoonotic disease with a wide distribution and a known public health significance caused by *Y. enterocolitica*.

One hundred and sixty marketable milk samples and some milk products including kareish cheese, Domiati cheese and small-scale ice cream were collected randomly from supermarkets, dairy farms, dairy shops, and street vendors, in Assiut city, Egypt. Selective CIN (Cefsulodin-Irgasan-Novobiocin) agar plates were used after enriched by phosphate buffered saline (PBS) supplemented with novobiocin and cefsulodin and incubated at 30 °C for 24 to 48 hours. The suspected colonies were identified by biochemical tests.

A total of 33 isolates (20.63%) were identified as *Yersinia species*, representing *Y. enterocolitica* (47.5%), *Y. pseudotuberculosis* (7.5%), *Y. pestis* (17.5%) and *Y. intermedia* (10%).

Keywords: Cheese, Ice cream, Marketable milk, Selective media (CIN), *Yersinia species*.

Introduction

Foodborne diseases (FBD) are continuing to be global public health trouble that has major effects on human health. On average 600 million people, almost 1 in 10 people in the world, fall ill at an annual rate after consuming contaminated food. Yersiniosis is considered the third-largest foodborne disease in the European Union caused by *Yersinia enterocolitica* bacteria (EFSA, 2015).

Yersinia species are members of the *Enterobacteriaceae*, these bacteria have Gram-negative or Gram-variable, rod-shaped or coccoid cells. Non-spore-forming rods, oxidase negative and catalase positive (Romero Cabello, 2007). Each *Yersinia* species, other than *Y. pestis*, is motile at 22–30 ° C but not at 37° C. Motile cells are always flagellated. *Yersinia* is capable of surviving in both aerobic and anaerobic culture conditions. It can grow between the temperatures of 0 and 45° C, being Optimum at 25–28° C, it can grow on non-selective and certain selective media (Dekker and Frank, 2015).

Currently, the genus *Yersinia* includes 19 established species (Nguyen *et al*., 2019). *Y. enterocolitica*, *Y. pseudotuberculosis and Y. pestis* are the only 3 pathogenic to humans. The remaining 16 are nonpathogenic; *Y. frederiksenii*, *Y. intermedia*, *Y. kristensenii*, *Y. nurmii*, *Y. bercovieri*, *Y. mollaretii*, *Y. canariae*, *Y. rohdei*, *Y. ruckeri*, *Y. aldovae*, *Y. aleksiciae*, *Y. entomophaga*, *Y. massiliensis*, *Y. pekkanenii*, *Y. similis, and Y. wautersii*, which are very common isolated from the environment (Redriksson and Laukkanen, 2018). The genus *Yersinia* is particularly important due to its ability to grow at low temperatures (4°C), so this psychrotrophic bacteria is considered a potential risk factor for the food cold chain (Soltan Dallal *et al.*, 2015). *Y. enterocolitica* was discovered more than 60 years ago but was not predicted to be a human or veterinary pathogen until the late 1960s, from that time it became more and more recognized in foodborne gastrointestinal infections (Sabina *et al.*, 2011).

It is transmitted to humans through the consumption of contaminated, milk (Thoerner *et al.*, 2003), and can

enter the milk from faces, bedding and improperly cleaned teats and milk handling equipment contaminated with soil or water (Sharma *et al.*,2003). Milk is an excellent source of nutrients for your body and is considered the most balanced food that humans consumed at any age in their life (Giannino *et al*., 2009). It has been classified as a major vehicle for foodborne pathogens in humans (Soltan-Dallal *et al.*, 2004). *Y. enterocolitica* is associated with many clinical and immunological manifestations, involving enterocolitis with diarrhea may be bloody in severe cases in infected infants and young children, but in older children and young adults, the clinical signs include acute terminal ileitis, pseudo appendicitis and mesenteric lymphadenitis. The extraintestinal manifestations are rarely pneumonia, reactive arthritis, erythema, mycotic aneurysm, axillary abscesses, and endocarditis (Menzies, 2010). Yersiniosis is a zoonotic disease with a wide distribution and a known public health significance; it is one of the most frequently reported zoonoses in the European Union caused by *Y. enterocolitica* (EFSA, 2009).

Materials and Methods

Sampling

One hundred and sixty marketable milk samples and some milk products including kareish cheese, Domiati cheese and small-scale ice cream (40 samples each) were collected randomly from supermarkets, dairy farms, dairy shops, and street vendors, in Assiut city, Egypt. The collected samples were transported to the laboratory in an insulated ice box to be examined bacteriologically. The samples were prepared according to A.P.H.A (2004).

Isolation and identification of *Yersinia* species

Enrichment procedure

One ml of each tube prepared with the sample from marketable milk or milk products was enriched by 10 ml of phosphate-buffered saline (PBS) supplemented with novobiocin and cefsulodin. The inoculated enrichment broth tubes were incubated at 4°C for 14 to 21 days (Soltan-Dallal *et al*., 2004).

Plating on selective agar media

A loopful from the liquid enrichment culture was streaked onto the selective CIN (Cefsulodin-Irgasan-Novobiocin) agar plates. The inoculated plates were incubated at 25◦C for 48 hours (Saberianpour *et al.*, 2012). The colonies which appeared with a deep red center (<1mm diameter) and surrounded by a transparent pale border (bull's eye-like) were subcultured on nutrient agar slants and incubated at 30°C for 24 hours. The slants were kept in a refractor (Sihvonen *et al.*, 2009).

Suspected colonies were identified by Gram stain. Yersinia species are Gram-negative coccobacilli with short rod-shaped cells. Biochemical tests such as catalase , oxidase, motility test, urease, and sugar fermentation tests were used for the identification of different *Yersinia* species (Dike and Bari, 2019).

Results

Table 1. Occurrence of *Yersinia species* in the examined samples.

Examined samples	No. of examined samples	Positive samples	
		No.	%
Marketable milk	40	24	60
Kareish cheese	40	1	2.5
Domiati cheese	40	-	0
Small-scale ice cream	40	8	20
Total	160	33	20.63

Table 2. Occurrence of different *Yersinia species* in the examined samples**.**

	Yersinia spp.							
Examined	enterocolitica		pseudotuberculosis		pestis		intermedia	
Samples	No./40	%	No./40	%	No./40	%	No./40	%
Marketable milk	15	37.5	3	7.5	3	7.5	3	7.5
Kareish cheese	1	2.5	-	0	-	0	-	0
Domiati cheese	-	0	-	0	-	0	-	0
Small-scale ice cream	3	7.5	-	0	4	10	1	2.5
Total	19	47.5	3	7.5	7	17.5	4	10

Discussion

Yersiniosis is one of the most frequently reported zoonotic diseases in the European Union (EFSA, 2009). *Y. enterocolitica* has a special emphasis on public health importance, because of its capacity in growing at refrigeration temperatures for a long time. Results given in Table1 showed that 20.63% (33 out of 160) of the tested marketable milk and milk products harbored *Yersinia species*. Among the 160 samples, 24 (60%) of marketable milk samples, 1 (2.5%) of kareish samples and 8 (20%) of small-scale ice cream samples were contaminated with Yersinia species, while failed to be detected in Domiati cheese. The highest isolation rate was 60% from marketable milk, followed by 20% from ice cream and 2.5% from kareish cheese, while couldn't be detected from Domiati cheese.

In the examined marketable milk samples, the lower results were recorded by Hussein and Ahmed (2002) 4%, Basyoni and Elsheikh (2005) 23.33%, Basha *et al.* (2008) 20%, Gűven *et al*. (2010) 1.33%, Darwish *et al.* (2015) 38.5 %, and Jamali *et al.* (2015) 6.5%.

In Kareish cheese, nearly similar results were recorded by Hassan and Afify (2007) 2%, while the higher results were obtained by Basyoni and Elsheikh (2005) 16.67%, and Basha *et al.* (2008) 14%. On the other side, in Domiati cheese, similar results were recorded by Basha *et al.* (2008) who failed to detect *Y. enterocolitica* in any samples of Domiati cheese.

In small-scale ice cream, the lower results were carried out by Khalifa *et al.* (2007) 12.5, Gűven *et al.* (2010) 2.67%, while the higher results were recorded by Al-Shammary and Madi (2016) 30%. The occurrence of Yersinia in small-scale ice cream may be because it is a psychrotrophic bacteria that is considered a potential risk factor for the food cold chain Soltan Dallal *et al.* (2015).

It is obvious that *Yersinia* species are isolated from milk in a high isolation rate (60%), and inadequate hygiene in dairy farms, particularly during milking could be the source of *Yersinia* species in raw milk Hanifian and Khani (2012). Poor hygienic measures during the production of milk products are probably the source of Yersinia species in milk products. The failure to detect this organism in Domiati cheese may be due to the higher percentage of salt usually added during manufacture. The collection of Kareish cheese from local markets and street vendors may explain the poor sanitary quality Basha *et al.* (2008).

Table 2 showed that *Y. enterocolitica* was found in 15 (37.5%), 1 (2.5%), 3 (7.5%) marketable milk, Kareish cheese and Domiati cheese samples respectively, while *Y. pseudotuberculosis* was detected in 3 (7.5%) of marketable milk samples only, further *Y. pestis* and *Y. intermedia* were found in 3 (7.5%) and 1 (2.5%) of marketable milk and small-scale ice cream samples respectively.

Various studies have stated the presence of *Y. enterocolitica* in milk and dairy products Myers *et al*. (2006). It has a special emphasis on public health importance because of its ability to grow in marketable milk at refrigeration temperatures for a long time, it probably has an impact on its frequency, and this is a serious issue for the food industry. The contaminated milk and dairy products that are consumed by humans have a higher opportunity of infection by *Y. enterocolitica* humans. It is an important zoonotic pathogen that can lead to Yersiniosis which is responsible for gastroenteritis and other syndromes Yucel and Ulusoy (2006).

Conclusion

Proper sanitation at all stages of handling and processing milk and dairy products is very important to be controlled from the occurrence of foodborne Yersiniosis. It can be easily destroyed by heat or pasteurization, so the pasteurization of milk is required before consumption and product making.

Conflict of interest

The authors haven't conflict of interest to declare.

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